



Investigations of Phytochemical and Antibacterial Activity of *Eucalyptus globulus* Leaves from Al-Khums, Libya

Salem Edrah^{1*}, Soad Belhaj², Afifa Omeman², Fouzy Alafid³ and Sumaia Aljarai¹

¹Department of Chemistry, Faculty of Sciences Al-Khums El-Mergib University Al-Khums Libya

²Department of Chemistry, Faculty of Education, El-Mergib University, Al-Khums, Libya

³Institute of Organic Chemistry and Technology, Faculty of Chemical Technology, University of Pardubice, Czech Republic

*Corresponding Author: Salem Edrah, Department of Chemistry, Faculty of Sciences Al-Khums El-Mergib University Al-Khums Libya.

Received: February 10, 2020

Published: March 17, 2020

© All rights are reserved by Salem Edrah, et al.

Abstract

Phytochemical constitute an essential interactive group of natural products in aromatic medicinal plants. *Eucalyptus globulus* was utilized as a medicinal plant for centuries due to its diverse characteristics. In this study, dried fresh leaves of *Eucalyptus globulus* were extracted being soaked in distilled water and ethanol, separately, for doing phytochemical screening and checking antimicrobial activity. Qualitative analysis revealed that steroids, sterols and triterpenes, carbohydrates, flavonoids, saponins, alkaloids, tannins and phenols, proteins, and amino acids gave positive results, whereas the anthraquinones gave negative results, in water crude extracts. While, with the quantitative analysis, the percentage yields of each of the chemical constituents that existing in *Eucalyptus globulus* leaves were 90 and 94% of aqueous and ethanolic extracts, respectively, additionally, percentage yield of each of alkaloids, Flavonoids, Saponins and Phenols 64, 59, 55 and 63%, correspondingly. Furthermore, the crude extracts of *Eucalyptus globulus* were tested using the Disc Diffusion Method to check their antimicrobial activity against bacterial pathogens. And the results of the effects of aqueous and ethanol extracts against these selected species of bacteria, where the maximum antibacterial activities of *Eucalyptus globulus* leaves aqueous and ethanol extracts, were observed 13, 12mm against *Klebsiella pneumonia*, separately, followed by also 12mm which formed from ethanol extract against *Staphylococcus epidermis*, and 11mm was formed from the aqueous extract of *Eucalyptus globulus* against *Staphylococcus epidermis* and 10mm was formed from ethanol extract against *Escherichia coli* then 9mm was formed from aqueous extract against *Escherichia coli*. After that 9 mm was formed from ethanol extract against *staphylococcus aureus* and 8 mm was formed from the aqueous extract of *Eucalyptus globulus* against *staphylococcus aureus*.

Keywords: *Eucalyptus globulus*; Chemical Constituents; Qualitative and Quantitative Analysis; Antibacterial Activities

Introduction

Therapeutic plants are beneficial for human beings and include an extensive variety of phytochemicals or bioactive composites that are employed to treat various ailments [1-3]. These medicinal plants are used in folk medicine, and studying such plants may

result in the discovery of novel and effective compounds against pathogenic microbes. Nowadays miscellaneous usage of synthetic drugs leads to countless side effects and this converge an idea to use medicinal plants as an alternative [4]. The plant-based technique of medicine being natural does not cause any serious side

effects or problems [5]. Almost all parts of plants used to treat various diseases, which are known to have diverse medicinal properties [6]. During the last three decades, numerous antibiotics produced, but the clinical efficiency of these existing antibiotics being threatened by the emergence of multidrug-resistant pathogens [7]. According to the World Health Organization (WHO), it's reported that medicinal plants would be the best source to obtain a variety of drugs [8]. Antibiotic resistance has become a global concern in recent decades and there is a growing concern regarding the search for new antimicrobial substances from different sources of medicinal plants [9]. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes [10]. *Eucalyptus globulus*, an important medicinal plant of *Myrtaceae* used in many parts of the world for the treatment of a wide variety of diseases. It acts as an antiseptic agent, and its leaf extracts used to treat influenza, skin rashes, chest problems and its vapor could be inhaled to fight inflammation. It stimulates the release of saliva and helps loosen a cough, it is used to treat lung infections and gastrointestinal ulcers. *Eucalyptus globulus* oil has useful antipyretic properties to reduce fever and decrease blood sugar levels. *Eucalyptus globules* also contain many aromatic principles that find out its existence in the composition of many products such as shampoos, toothpaste, soaps, ointments, air fresheners etc. [11]. In addition, studies on medicinal plants in Libya are very rare, principally for this plant, including benefiting, acquiring and more knowledge about it. Which may serve this plant in the Pharmaceutical industry for the use of it as a treatment of diverse sorts of diseases. This study aims to investigate of phytochemical constituents in aqueous and ethanolic crude extracts of *Eucalyptus globulus* leaves collected from Al-Khums region, Alkhums, Libya, additionally, screen these crude extracts against to some species of humane pathogenic bacteria.

Materials and Methods

Plant material

Fresh healthy samples of *Eucalyptus globulus* leaves were collected from the countryside of Al-Khums, Al-Khums, Libya. Samples were washed by tap water and then by distilled water and they were next dried in the shade; the rest of the drying process was done in an oven at 42 - 43 °C. Samples were powdered by an electrical blender and were kept in dark, airtight bottles until further usage.

Extraction

20 g of fine powdered *Eucalyptus globulus* leaves, was soaked in 400 ml of appropriate solvent (distilled water and ethanol, separately), and put into different flasks which were placed on the shaker and then left at a room temperature 25 ± 2 °C for 72 hours. The solvent was filtered and then removed using reduced pressure with the help of a rotary vacuum evaporator. this whole extraction process yielded a viscous dark brown residue of water extract and a viscous dark green residue of ethanol extract.

Phytochemical screening

Qualitative phytochemical screening

The finely powdered *Eucalyptus globulus* leaves and the prepared crude extracts had a phytochemical Screening carried out for the existence of bioactive composites via standard methods [12-16].

Detection of sterols and triterpenes

5 g of the finely powdered leaves were put in a test tube alongside 20 ml of 50% alcohol then the tube was heated for about 3 min in a water bath. It was then allowed to cool at room temperature to commence filtration. The filtrate was evaporated in an evaporating beaker until the beaker was completely dry, about 10 ml of petroleum ether was added to the beaker and stirred for 5 min, the petroleum ether portion was then discarded. And 15 ml of chloroform was then added and stirred for about 5 min, it then was transferred into a test tube, and about 1 mg of anhydrous sodium sulfate was added to it and gently shaken and filtered, and the filtrate was then divided into two test tubes and used for the following tests:

- **Salkowski's test:** In the first test tube: 2 to 3 drops of concentrated Sulphuric acid were added in order to form a lower layer. The reddish-brown color at the interphase indicates the existence of a steroidal ring.
- **Lieberman-burckhardt's reaction:** In the second test tube: an equal volume of acetic anhydride was added and then gently mixed. Then 1 ml of concentrated H_2SO_4 added down the side of the tube. The appearance of a brownish-red ring at the contact zone of the two liquids and a greenish color in the separation layer indicates the existence of sterols and triterpenes.

Testing for carbohydrates

- **Fehling's test:** 2ml of Fehling A and B reagents (Equal volume mixed together) added to 5 ml of crude extract and gently boiled. A brick-red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.
- **Benedict's:** 2ml of Benedict's reagent mixed with 5 ml of crude extract and boiled; a reddish-brown precipitate formed which indicated the presence of the carbohydrates.
- **Molisch's test:** 2ml of Molisch's reagent mixed with 5 ml of Crude extract and the mixture shaken accurately. Afterward, 2ml of concentrated H_2SO_4 poured prudently along the side of the test tube. The appearance of a violet ring at the interphase indicated the presence of carbohydrates.
- **Iodine test:** 5 ml of the crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated that carbohydrates were present.

Testing for flavonoids

- **Shinoda test:** 5 ml of the crude extract was mixed with a few fragments of magnesium powder. Concentrated HCl was added dropwise. The pink scarlet color appeared after a few minutes, which indicated the presence of flavonoids.
- **Alkaline reagent test:** 5 ml of the crude extract was mixed with 2ml of 2% NaOH solution. An intense yellow color formed which turned colorless after adding a couple drops of diluted HCl acid which indicated the presence of flavonoids.

Detection of saponins

5ml of distilled water was mixed with the extract in a test tube and then shaken vigorously; the formation of stable foam is taken as an indication for saponins presence.

Detection of alkaloids

5 g of finely powdered leaves were boiled in a water bath with 50 ml of 5% H_2SO_4 in 50% ethanol. Then it was cooled and filtered. A portion of it was set aside. One more portion of the filtrate was put in 200 ml separating funnel and the solution was made alkaline by adding 3 drops of concentrated ammonia solution, for forming, an equal volume of chloroform was added and shaken gently to allow the layers to separate. The lower chloroform layer was col-

lected into a second separating funnel. The ammoniacal layer was set aside for usage later. The chloroform layer extracted with two quantities each of about 15 ml of dilute Sulphuric acid. The various extracts were then used for the following test:

- **Wagner's test:** 2 ml of the filtrate was added to 1 ml of Wagner's reagent in dropwise. The formation of a reddish-brown precipitate indicates the presence of alkaloids.
- **Dragendorff's test:** 2 ml of the filtrate added in 1 ml of Dragendorff's reagent in dropwise. The formation of a reddish-brown precipitate indicates the presence of alkaloids.
- **Mayer's test:** 2 ml of the filtrate added in 1 ml of Mayer's reagent in dropwise. The formation of a greenish colored or cream precipitate indicates the presence of alkaloids.

Detection of tannins and phenols

5 ml of the crude extract mixed with 1ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Detection of proteins and amino acids

- **Millon's test:** 5 ml of crude extract was mixed with 2ml of Millon's reagent; a white precipitate appeared which turned red upon being gently heated, which indicates the presence of protein.
- **Ninhydrin test:** 5 ml of crude extract was boiled with 2ml of 0.2% solution of Ninhydrin, a violet color appeared to indicate the presence of amino acids and proteins.

Detection of anthraquinones

0.5 g of the crude extract dissolved in 5 ml of 1% HCl and boiled then filtered. The filtrate was shaken with 5 ml benzene and the benzene layer was decanted, 10% Ammonium hydroxide added and the color in the alkaline phase observed. The formation of pink-violet or red color indicated the presence of Anthraquinones.

Quantities screening

Quantitative Analysis for the alkaloids, Flavonoid, Saponins and total phenols that present in the extracts carried out by following the procedures: [16-21].

Alkaloid determination

10g of finely powdered leaves weighed whilst inside a 500 ml beaker and added to it 400 ml of 10% acetic acid in ethanol added,

covered, and left alone for 4 h. This filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide added dropwise to the extract until the precipitation was complete. The whole solution was left alone, the precipitated was collected and washed with dilute ammonium hydroxide and then it was filtered. The residue is the alkaloid, which was dried and weighed [17].

Flavonoid determination

5g of finely powdered leaves was extracted using 50 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper 125 mm. The filtrate was then later transferred into a crucible, evaporated to dryness inside a water bath, and weighed to a constant weight [18].

Saponins determination

10g of plant sample was mixed with 100 ml of 20% ethyl alcohol. The suspension heated over a water bath for 4 h with continuous stirring at about 50°C. The mixture was then filtered and the residue re-extracted with another 100 ml of 20% ethyl alcohol. The combined extracts reduced to 20 ml over a water bath at about 80°C. The concentrate was then transferred into a 250 ml separating funnel and about 10 ml of diethyl ether added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification procedure was repeated. 30 ml of n-butanol extracts were washed twice using 5 ml of 5% aqueous sodium chloride. The remaining solution was then heated in a water bath. After evaporation, the sample was dried in the oven into a constant weight. The saponins content is written in percentage [19].

Total phenols determination

10 g fine powdered sample weighed whilst inside a 500 ml titration flask and 200 ml n-hexane was added twice and left for 4 h each; the filtrates were discarded for a fat-free sample preparation. Then 100 ml of diethyl ether added twice, heated for 15 min each, then cooled up to room temperature and filtered into a separating funnel. About 100 ml of the 10% NaOH solution added twice and shook well each time to separate the aqueous layer from the organic layer. It was washed three times with 50 ml deionized water. The total aqueous layer acidified up to pH 4.0 by adding 10% HCl solution and 100 ml dichloromethane (DCM) twice to acidify the aqueous layer in the separating flask. Consequently, the organic layer was collected, dried and then weighed [20-21].

Antibacterial activity assaying

The aqueous and ethanolic crude extracts of the *Eucalyptus globulus* were tested against four strains of human pathogenic bacteria obtained from Analysis Laboratory of Al-Khums Teaching Hospital, Al-Khums Libya, intended for the examinations of two strains of gram-positive bacteria include *Staphylococcus aureus* and *Staphylococcus epidermis*, and two strains of gram-negative bacteria including *Escherichia coli* and *Klebsiella pneumonia*, the sensitivity of the microorganisms to the extracts of bout plant's species was measured and compared with each other and with designated antibiotics the measurement was conducted by checking the diameter of the inhibition zones. The antibiotic discs such as Ciprofloxacin (Cipro.) and Tetracycline (Tetra) were placed on the surface of the plates to be used as positive control while the aqueous and ethanol solvent used as a negative control. The plates were incubated at 37°C for 24 hours and after incubation, the diameter of the inhibition zones was measured in mm and recorded), (Sterilized discs paper 6 mm in size, and each sterile disc was saturated separately with 50 µl of crude extracts using micropipette, and they were allowed to dry, then immediately placed into Muller Hinton Agar plates were prepared and cultivated by the appropriate test organisms were swabbed over the surface of agar plates using sterile cotton swab), After incubation for 24 hours at 37 °C the diameter of inhibitory zones formed around each disc were measured in millimeter (mm) and recorded, a good inhibition of more than 6 mm were observed indicating the effective antibacterial activity of the bioactive constituents [22,23].

Results and Discussion

Quantitative analysis

As shown in table 1 the percentage yields of each chemical constituent's present in *Eucalyptus globulus* leaves were 90 and 94% of aqueous and ethanolic extracts, respectively, while percentage yields of each alkaloid, Flavonoids, Saponins, and Phenols in *Eucalyptus globulus* were 64, 59, 55 and 63%, correspondingly. These results reflect the importance of this plant in folk medical use for disease treatment and give importance nowadays to considering it as a source for extracting these chemical constituents as a benefit in drug manufacturing. In addition, it provides a moreover conception for a specific investigation to propose some biochemical basis for ethno-pharmacological utilization of this specie in the treatment of many diseases such as diabetes [24].

Plants Names	Percentage Yields (%)					
<i>Eucalyptus globulus</i>	Chemical Constituents		Alkaloids	Flavonoids	Saponins	Phenols
	Aqueous	Ethanollic				
	90	94	64	59	55	63

Table 1: Results of the Quantitative analysis of the *Eucalyptus globulus*.

Qualitative analysis

Table 2 shows the phytochemical screening results of several chemical constituents from the picked plant, where through the qualitative analysis, revealed that the presence of all composites such as Steroid, Sterols and Triterpenes, Carbohydrates, Flavonoids, Saponins, Alkaloids, Tannins and Phenols, Proteins, and amino acids, Anthraquinones was not present in the crude extracts (aqueous and ethanolic) of leaves of *Eucalyptus globulus*. As for the known antimicrobial activities of these phytochemical composites, their presence in the extract is reasonable, and also the reason for the antimicrobial effects exhibited by this plant; on *Staphylococcus aureus* and *Salmonella typhi* (*S. typhi*). And these phytochemical constituents are known to have antimicrobial effects, Tannins, for instance, recognized to be formed up from phenolic compounds

and phenols, and phenolic compounds have been used extensively as disinfectants. The action of tannin may be due to protein denaturation and found to be non-specific [25]. Tannins possess astringent and homeostatic properties and therefore widely used as a topical application on sprains, bruises, surface wounds and infections. Phytochemical screening of the extracts varies from one plant to another from the same species, which means that they could also vary from place to place due to geographical location, climatic circumstances and soil condition of their resident area. This may explain why it could be reasonable to have variations for the chemical constituents with our studied plant result when compared to another researched plant in other areas. Furthermore, from the results reported in the literature, propose that some species interact in highly active against bacteria, as antimalarial and influenza viruses [26].

Name of the compound	Crude extracts of leaves of <i>Eucalyptus globulus</i>		
	H ₂ O	EtOH	Used reagents, results
Tannins and phenols	+	+	Ferric Chloride: Blue- Green or Black
Saponins	+	+	Foam: Stable Foam
Flavonoids	+	+	Shinoda: Pink Scarlet
	+	+	Alkaline Reagent: Yellow
Carbohydrates	+	+	Fehling: Brick red precipitate
	-	-	Benedict: Reddish Brown Precipitate
	+	+	Mulish: Violet Ring
	+	+	Iodine: Dark Blue or Purple
Sterols and Triterpenes	+	+	Salkowski's :Reddish-brown
	+	+	Lieberman-Burckhardt: Brownish-Red ring and green
Alkaloids	+	+	Wagner: Reddish-Brown Precipitate
	+	+	Dragendorff's: Reddish-Brown Precipitate
	+	+	Mayer's: Creamy Precipitate
Anthraquinones	-	+	Ammonium hydroxide: Pink-Violet or Red
Steroid	+	+	Chloroform: Red
Proteins and amino acids	-	-	Millon's: Red
	+	+	Ninhydrin: Violet

Table 2: Phytochemical screening results of crude extracts of leaves of *Eucalyptus globulus*:

+ = Present, - = Absent, EtOH = Ethyl Alcohol, H₂O = Distilled water.

Antibacterial activity

As shown in table 3, the results of this study revealed that the crude extracts (aqueous and ethanolic) of *Eucalyptus globulus* acted to perceived inhibit the growth of selected pathogenic bacteria, this investigation against some specific of humane pathogenic bacteria, where the maximum antibacterial activities of *Eucalyptus globulus* leaves aqueous and ethanol extracts, were observed 13, 12mm against *Klebsiella pneumonia*, separately. Followed by also 12mm formed from ethanol extract against *Staphylococcus epidermis*, and 11mm was formed from the aqueous extract of *Eucalyptus globulus* against *Staphylococcus epidermis* and 10mm was ethanol extract against *Escherichia coli* then 9mm aqueous extract against *Escherichia coli*. followed by 9 mm was from ethanol extract against *staphylococcus aureus* and 8 mm was from the aqueous extract of *Eucalyptus globulus* against *staphylococcus aureus*. Nevertheless, the greatest activity demonstrated by the standard antibiotics Tetracycline and Ciprofloxacin (control), this could be because the antibiotic is in its pure state. In Libya, the application of therapeutic plants particularly in traditional medicine currently well recommended. Moreover, the increasing resistance of the largest synthetically produced antimicrobial agents is of most anxiety. Hence, proper medicinal plants possessing sufficient remedy confidence that is inadequate damaging to human tissue and which necessitates studying it. Utilizing of *Eucalyptus* extracts such oil from the leaves and can be applied for disinfection, cleaning, offers antiseptic properties [27], and in food additives is extremely precise quantities, mainly desserts, decongestant and cough droplets [28]. In addition, some kinds of *Eucalyptus* such as *globules* have an inhibition influence toward some Gram-positive bacteria [28].

Bacterial Name	Plant Leaves Extracts (mm)		Antibiotic (mm)	
	Aqus.	EtOH	Tetra	Cipro
<i>Escherichia coli</i>	9	10	19	20
<i>Staphylococcus aureus</i>	8	9	18	19
<i>Staphylococcus epidermis</i>	11	12	21	18
<i>Klebsiella pneumonia</i>	13	12	22	17

Table 3: Results of Anti-bacterial activity assay of the leaves of the *Eucalyptus globulus* extracts:

(mm) = millimeters, Tetra = Tetracycline, Cipro = Ciprofloxacin.

Conclusion

The phytochemical ingredients of the *Eucalyptus globulus* leaves were examined, Steroid, Sterols and Triterpenes, Carbohydrates, Flavonoids, Saponins, Alkaloids, Tannins and Phenols, Proteins, amino acids, and Anthraquinones were detected in this plant leaves extracts. In addition, these very extracts were tested against the pathogenic humane bacteria and showed a good effect which encourages the use of this plant for medication against several types of diseases. These results, also, confirm that the potent use of *Eucalyptus globules* in the curative pharmaceutical manufacturer, which might be beneficial as an alternative antimicrobial agent in indigenous medication for the remediation of several diseases.

Bibliography

1. Kiruba S., et al. "Phytochemical analysis of the flower extracts of *Rhododendron arboretum* Sm. Ssp. *Nilagiricum* (Zenker) Tagg". *Asian Pacific Journal of Tropical Biomedicine* 5 (2011): 284-286.
2. Rao TG., et al. "Anti-microbial principles of selected remedial plants from Southern India". *Asian Pacific Journal of Tropical Biomedicine* 1 (2011): 298-305.
3. Raja ARD., et al. "Antibacterial activity of selected ethnomedicinal plants from South India". *Asian Pacific Journal of Tropical Medicine* 4 (2011): 375-378.
4. Nostro A., et al. "Extraction methods and bioautography for evaluation of medicinal plants antimicrobial activity". *Letters in Applied Microbiology* 3 (2000): 379-384.
5. Ayyanar I. "Herbal medicines of wound healing among tribal people in Southern India: Ethnobotanical and Scientific Evidence". *International Journal of Applied Research in Natural Products* 2 (2009): 29-42.
6. Chakraborty A and Brantner AH. "Antibacterial activity of the stem bark of *Holarrhenapubescens* (Syn. *H. antidysenterica*)". *Journal of Ethnopharmacology* 68 (1999): 339.
7. Bandow JE., et al. "Proteomic approach to understanding antibiotic action". *Antimicrob Agents Chemother* 47 (2003): 948-955.
8. Santos PRV., et al. "Control microbiogicodeproductos. Fitoterapicos". *Revista de Farmacia e Bioquímica* 31 (1995): 35-38.
9. Westh H., et al. "Sarisa study group: an international multi-center study of antimicrobial consumption and resistance in *Staphylococcus aureus*". *Microbial Drug Resistance* 10 (2004): 169-176.

10. Afloyan AJ. "Extracts from the shoots of *Arctotis arctotoides* inhibit the growth of bacteria and fungi". *Pharmaceutical Biology* 41 (2003): 22-25.
11. Musyimi DM and Ogur JA. "Comparative assessment of anti-fungal activity of extracts from *Eucalyptus globulus* and *Eucalyptus citriodora*". *Research Journal of Phytochemistry* 2 (2008): 35-43.
12. Sofowra A. "Medicinal Plants and Traditional Medicine in Africa". Spectrum Books Ltd., Ibadan, Nigeria (1993): 191-289.
13. Trease GE and Evans WC. "Pharmacognosy, 11th edition". Bailliere Tindall, London (1989): 45-50.
14. Harborne JB. "Phytochemicals Methods". Chapman and Hall Ltd., London (1973): 49-188.
15. Ramasamy Thangavelu Narendhirakannan., *et al.* "Evaluation of antibacterial, antioxidant and wound healing properties of seven traditional medicinal plants from India in experimental animals". *Asian Pacific Journal of Tropical Biomedicine* (2012): S1245- S1253.
16. Trease and Evans WC. *Pharmacognosy*. 15th. Edition, Elsevier, India (2002).
17. Harborne JB. "Phytochemical methods, London, Chapman and Hall, Ltd (1973): 49-188.
18. Boham AB and Kocipai AC. "Flavonoid and condensed tannins from Leaves of Hawaiian *vaccinium vaticulum* and *vicalycinium*". *Pacific Science* 48 (1994): 458-463.
19. Nahapetian A and Bassir A. "Changes in concentration and interrelationships of phytate, P, Mg, Cu, Zn in wheat during maturation". *Journal of Agricultural and Food Chemistry* 32 (1975): 1179-1182.
20. Obadoni BO and Ochuko PO. "Phytochemical studies and comparative efficacy of the crude extracts of some hemostatic plants in Edo and Delta states of Nigeria". *Global Journal of Pure and Applied Sciences* 8 (2002): 203-208.
21. Raaman N. *Phytochemical techniques*, New India Publishing Company, New Delhi (2006): 19-22.
22. Harauma A., *et al.* "Mulberry leaf powder prevents atherosclerosis in apolipoprotein E-deficient mice". *Biochemical and Biophysical Research Communications* 358 (2007): 751-756.
23. Cordell GA., *et al.* "The potential of alkaloids in drug discovery". *Phytotherapy Research* 15 (2001):183-205.
24. Ogunwande IA., *et al.* "Aromatic Plants growing in Nigeria: Essential Oil Constituents of *Cassia alata* (Linn.) Roxb and *Helianthus annuus* L". *Records of Natural Products* 4.4 (2010): 211-217.
25. Ody P. "The Complete Medicinal Herbal". Dorling Kindersley, London (1994): 61.
26. Gupta S., *et al.* "In-Vitro antioxidant and Free Radical Scavenging Activities of *Ocimum Sanctum*". *World Journal of Pharmaceutical Research* 1 (2012): 78- 94.
27. Garcia MD., *et al.* "Antinociceptive and anti-inflammatory effect of the aqueous extract from leaves of *Pimentaracemosavar. ozua* (Myrtaceae)". *Journal of Ethnopharmacology* 91.1 (2004): 69-73.
28. Igbe I., *et al.* "Anti-inflammatory activity of aqueous fruit pulp extract of *Hunteria umbellata* K. Schum in acute and chronic inflammation". *Acta Poloniae Pharmaceutica* 67.1 (2010): 81-85.

Assets from publication with us

- Prompt Acknowledgement after receiving the article
- Thorough Double blinded peer review
- Rapid Publication
- Issue of Publication Certificate
- High visibility of your Published work

Website: www.actascientific.com/

Submit Article: www.actascientific.com/submission.php

Email us: editor@actascientific.com

Contact us: +91 9182824667